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EDITORIAL

From the ureteric bud to the penome

The kidney collecting system arises through branching morphogenesis of the ureteric bud (UB). Key signals regulating the process are derived from the surrounding metanephric mesenchyme and stroma. After many iterations of branching, the tree that becomes the collecting system is formed [1]. Aberrations are thought to alter nephron number, possibly predisposing to hypertension and progressive renal disease [2]. The branching process may be exploitable for tissue engineering purposes [3]. As data accumulates, the clinical ramifications of branching are becoming broader and broader.

It is customary to divide collecting system development into stages, but different investigators have parsed them differently. This is not the place to argue why the particular division that follows makes more sense than others; suffice it to say that it seems reasonable to conceive of a staged process consisting of ureteric bud outgrowth (stage 1), rapid branching (stage 2), a slowing down of branching (stage 3), and the stopping of branching and tubular differentiation/maintenance (stage 4). In this scheme, nearly all branching occurs in stage 2 and stage 3. Key questions for the field include: What is the molecular basis of the switching mechanism between each stage? And where do all the genes that mutational and in vitro studies have implicated as important for collecting system development—like the gene described in this issue of *Kidney International* by Araki et al [4]—fit into this scheme?

Although much work still needs to be done, a few generalizations can be attempted. In vitro studies strongly suggest that growth factors (acting singly or in combination) can, in the appropriate extracellular matrix context, function as switches that move the developing tree from one stage to the next. And if one views the genetic circuitry of UB branching as a network of linked nodes (e.g., an airline route map), mutational data in both mouse and humans suggest that certain genetic pathways constitute hubs of varying importance [5]. For example, for stage 1 (UB outgrowth), the glia-derived neurotrophic factor (GDNF)-ret axis (together with interacting genes) is the equivalent of Chicago's O'Hare Airport—with all major airlines routed through this hub because disruption of this pathway leads to renal agenesis. In stage 4 (differentiation/maintenance), recent data on cystic disease are consistent with the idea that the genes regulating ciliary morphogenesis and function are something of a minor hub—"minor" because there seem to be other important parallel pathways that are involved in stage 4 morphogenesis. So, in contrast with the genes directly linked to the GDNF-ret axis in stage 1 (O'Hare), the ciliary genes in stage 4 are more like Las Vegas Airport—a place that many airlines travel to and one that is essential to the financial existence of some, but most can stay in business without going there.

What is the hub for phase 2 and phase 3? Or rather, what is the O'Hare or Las Vegas Airport for the network of genes that regulates branching? Perhaps there is none. Knockout data does not yet support the existence of such hubs, or at least major ones. In vitro data from cell culture, organ culture, and isolated UB culture are consistent with a variety of redundancy-type arguments, again suggesting that there is no bottleneck hub. Is it possible that the network is so decentralized that any in vivo disruption of individual nodes leads to rerouting around the disruption that does not ultimately affect the outcome in terms of the number of UB branching events (and thus nephron number)? That may well be. For stages 2 and 3, closing of the airport in Chicago during a blizzard does not prevent one from traveling between Los Angeles and New York City. And if Los Angeles Airport (LAX) is closed, one can fly out of Orange County. If both JFK and LaGuardia are closed, one can fly into Newark. And so on. For the routing map in stages 2 and 3 is very different from stage 1; there appear to be no "superhubs," but instead, dozens of equivalent nodes. The routing through O'Hare, LAX, and JFK is not very different from routing through the Minneapolis and Las Vegas airports, which might not be all that different from Albuquerque and Omaha and Fargo. This may be why studies have not detected a hub of comparable importance the GDNF-ret axis in stage 1, or even the ciliary genes of stage 4.

These generalizations seem to hold for stage 2 and stage 3 as long as one does not include genes such as signaling molecules that pleiotropically regulate many different basic cellular processes. About a decade ago, a number of intracellular kinases, including protein kinase C (PKC) and phosphoinusitide-3-kinase (PI3K), were implicated in the branching of cultured renal epithelial cells in three-dimensional matrices [6, 7]. Subsequent work in organ culture also implicated these genes in branching of the UB [8]. Because so many pathways feed through them, these kinases, in a sense, could be considered some sort of hub. But they are key to so

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many cellular processes necessary for UB growth and branching—motility, apoptosis, cell shape changes, proliferation, basement membrane interactions, and so on—that it is hard to think of them as true morphogenetic hubs that are relatively specific to stages 2 and 3 because they seem to be involved in just about everything. However, it is plausible that the search for genes regulated by these kinases may lead to the identification of important nodes and even hubs that are specific to stage 2 and stage 3 branching. Following the targets of essential kinases still makes a lot of sense.

This is what continues to make differential screening approaches like the one taken by Araki et al [4], which aim to identify genes expressed under various "plus-minus" conditions, interesting. Differential screening methods have been used to identify a number of genes that seem to be involved in branching. The question is: How involved is "involved?" That has been very hard to sort out. As already discussed, one reason may be that there are many equivalent nodes in the branching process and no real hubs. Part of the problem is the difficulty in reconciling in vitro developmental biology with genetic approaches; part of the problem is also a battle of cultures between investigators who favor one type of approach over the other. Fortunately, with the growing realization that knockout and transgenic mice can shed only limited insight on many key problems in the field of nephrogenesis, the situation seems to be improving, if slowly.

Araki et al have found a new gene, metanephros derived tubulogenic factor (MTF), that is regulated by PKC during kidney development, and this gene appears to be involved in UB branching [4]. At the moment, it is unclear whether this is one of a large number of nodes involved in Stage 2 or Stage 3 branching, or something greater, but the approach of using differential screening to link one pathway to another could conceivably lead to the identification of some kind of branching hub, perhaps not equivalent to the GDNF-ret axis (O'Hare) in Stage 1, but then again, that awaits more detailed analysis of its role in branching.

Branching is so fascinating because it is integral to so many natural processes, living and nonliving. And one must not forget the prevalence of tree metaphors in visual art, mythology, poetry, and prose. Part of the attraction of trying to understand branching lies not only in the possibility of applying mathematics and physical science approaches to a process of tremendous biologic and clinical interest (i.e., systems biology); it is important to bear in mind that much of the allure, even for scientists (or perhaps especially for scientists), is metaphorical. In a recently published novel, a neurotic depressed scientist imagines "an infinite tree that he could climb and climb, following its endlessly bifurcating branches until he reached a fluffy cloud that would forever hide him from the rest of the world. [9]" Although active experimentalists do not generally talk about it publicly, either because there is no forum or because they are hesitant to come to terms with nonobjective factors involved in the scientific enterprise, one cannot discount the impact of such musings on the problem of scientific problem-finding and problem-solving.

The paper in the current issue of *Kidney International* adds yet another gene that must be considered as we develop models of the genetic circuitry of UB branching. The challenge is a big one. If a problem ever demanded a systems approach—in the broadest sense—branching is it. Work on the branching transcriptome is under way, as is mathematical modeling and the application of engineering approaches to biologic branching, but much of the preceding discussion is about something bigger that extends beyond the goal of systems biology—the so-called systeome. Rather, it might be termed a penultimateome—perhaps called the penome?—that seeks to explore the links between the sciences and humanities, not in an airy metaphysical manner, but by using the critical academic eye to examine the data, broadly construed, that exists in the sciences and arts, searching for interfaces, intersections, and possibly even general principles. How far can we go before it all gets too murky for our scientific taste? The long-sought unification of all fields, dubbed "consilience" a few years ago [10], seems too ambitious to this experimental nephrologist. But an intermediate step seems, at least at the moment, not only plausible but desirable. Is there some middle ground between waxing transcendent and detailed quantitative descriptions of reality—and if so, where is that ground? In other words, if we concede that the extreme ends of the arts and sciences may never meet, where in the spectrum is there a point that they do meet, and more importantly, how do we define it? Will the product of such an effort look more like a personal essay, a humanities paper, a scientific report or some kind of hybrid that constitutes a new form? Almost half a century after the famous two-culture debate began [11], where do we stand? We need to find out by delving deeply, broadly, and openmindedly into very specific problems in order to explore, using available approaches that are consistent with academic rigor, how far we can get. There is a lot to gain by focusing an abstract debate on a concrete problem, and the answers may prove surprising to many in both the sciences and arts. Branching is a ripe area for such an exploration.

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